The Phenotypic Spectrum of Histiocytosis X Cells

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Proliferating cells in histiocytosis X (histiocytosis X cells) share many structural and immunophenotypic features with Langerhans cells, leading to the assumption that histiocytosis X represents a proliferative disorder of Langerhans cells. Because, depending on their state of activation and/or differentiation, Langerhans cells exhibit a varying immunophenotype, we investigated whether histiocytosis X cells display a similar phenotypic heterogeneity and, if so, whether the heterogenous biological behavior of histiocytosis X is reflected by differences in the immunophenotype of the proliferating cells. In 21 patients suffering from different clinical manifestations of histiocytosis X, proliferating cells uniformly expressed class I and II alloantigens, T200, CD1, CD4, and S100 protein. In 12 of 21 cases, histiocytosis X cells additionally exhibited immunocytochemically detectable amounts of C3b and C3bi receptors and certain monocyte/macrophage antigens (CDw14, Ki-M1, Ki-M6). This immunophenotypic heterogeneity of histiocytosis X cells could not be correlated with clinical course, prognosis, and final outcome of the disease in a given patient. The capacity of histiocytosis X cells to immunophenotypically mimic various states of Langerhans cell activation and/or differentiation, however, underscores the concept of histiocytosis X as a proliferative disorder of Langerhans cell origin. *J Invest Dermatol 90:441–447, 1988*

Eosinophilic granuloma [1], Hand–Schüller–Christian disease [2], and Abt–Letterer–Siwe disease [3] were originally described as distinctive clinical entities characterized by a proliferation of histiocytic cells in one or more body tissues, resulting in either a rather benign (solitary lesions in one organ) or a fatal (multisystemic disease) clinical picture. These three disease states were reclassified under the unifying but rather unrevealing term *histiocytosis X* (HX) [4] mainly because in a given patient one occasionally encounters a transition from the benign variant to a more aggressive form of the disease. Though not unchallenged on clinical grounds [5], this unifying concept was further strengthened by the observation that the proliferating cells (HX cells) in lesions of patients in each of these three disease categories display the essential ultrastructural and immunophenotypic features of Langerhans cells (LCs), including an abundance of Birbeck granules and surface expression of class II and CD1 antigens [6–10]. These findings led to the concept that HX represents a proliferative disorder of LCs [11].

Prevailing opinion holds that LCs, residing primarily within stratified squamous epithelia, constitute a distinctive member within a family of bone marrow-derived Ia^+ dendritic cells (reviewed in Ref 12). Other members of this cell system supposedly include veiled cells, interdigitating cells (IDCs) (reviewed in Ref 13), and certain circulating mononuclear cells [14,15]. Although these various cell populations share a common and unique functional capacity, that is, elicitation of primary immune responses (reviewed in Refs 12 and 13), there exist certain phenotypic differences between the various family members which are listed in Table I. It has been further demonstrated that even in a given subpopula-

Table I. In Situ Immunophenotypes of Langerhans Cells (LCs), Veiled Cells (VCs), Interdigitating Cells (IDCs), and Circulating LC-Related Cells (cLC-rCs)

Antigen	LCs	VCs	IDCs	cLC-rCs
Class I	+ [16][a]	nd[b]	++ [17]	nd
Class II	++ [18,19]	++ [20]	++ [17]	++ [15]
T200 (CD45)	++ [21]	nd	++ [17]	nd
PNA[c]	+ [22]	nd	nd	+ [15]
CD1	++ [23,24]	++ [25]	++ [24]	++ [14,15]
CD4	± [27,28]	nd	++ [26]	nd
S-100	++ [29]	nd	++ [17]	nd
CR1	− [17,26]	− [20]	+[d]	nd
CR3 (CD11)	− [30]	nd	nd	nd
CDw14	− [17]	nd	+ [17]	+ [15]
Ki-M1	−[d]	nd	+ [31]	nd

[a] Reactivity with appropriate monoclonal antibody: −, negative; ±, weak; +, moderate; ++, strong.
[b] nd, not done.
[c] PNA, peanut agglutinin.
[d] Our observations.

Manuscript received July 8, 1987; accepted for publication October 15, 1987.

This work was supported, in part, by grant 4951 from the Fonds zur Förderung der wissenschaftlichen Forschung and by grants from the Österreichische Nationalbank and from the Bürgermeisterfonds der Stadt Wien, Vienna, Austria.

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Abbreviations:
DAB: diaminobenzidine
FITC: fluorescein isothiocyanate
HRPO: horseradish peroxidase
HX: histiocytosis X
IDC: interdigitating cells
LC: Langerhans cell
PBS: phosphate-buffered saline
PE: phycoerythrin
RT: room temperature

0022-202X/88/$03.50

tion of this cell family, the expression of certain surface antigens is not a stable trait. In particular, "resting" (i.e., in situ, freshly isolated) human LCs express small amounts of CD4 antigens [28] and fail to react with monoclonal antibodies directed against receptors for cleavage fragments of C3, certain monocyte/macrophage antigens, and interleukin-2 receptors [17,21,26,30,32,33]. Human LCs in diseased skin are characterized by their strong reactivity to anti-CD4 antibodies [28], and under tissue culture conditions, these cells lose their anti-CD4 reactivity (our observation), express myeloid differentiation antigens and complement receptors in immunocytochemically detectable amounts, increase their class II expression, and acquire interleukin-2 receptors [33,34]. Thus, assuming (1) that depending upon a given state of differentiation and/or activation, LCs and related cells may display a varying phenotype and (2) that HX indeed represents a proliferative disorder of LCs and/or related cells, we asked whether the heterogenous biological behavior of HX is reflected by differences in the immunophenotype of the proliferating cell.

MATERIAL AND METHODS

Subjects Our study included tissue specimens from 21 patients suffering from HX. Thirteen patients (8 males, 5 females) were part of a German–Austrian prospective cooperative multicenter study (DAL-HX 83) that started in June 1983. Their ages at diagnosis ranged from newborn to 15 years. These patients were carefully examined according to the diagnostic workup employed by the DAL-HX 83 protocol [35]. Diagnosis of organ involvement was based on clinical and laboratory parameters as well as biopsy findings. Organ dysfunction was diagnosed according to the criteria elaborated by Lahey in 1975 [36]. Patients were categorized into

Table II. Patients With Localized or Disseminated Histiocytosis X

Subject	Sex	Age at Diagnosis	Organ Involvement at Diagnosis	Classification[a] (DAL-HX 83)	Biopsy[b]	Therapy	Present State of the Disease
1[c]	M	3 months	Skull	Aa	Bone	Local management	No evidence of disease
2[c]	F	9 months	Skull	Aa	Bone		
3[c]	M	2 years	Skull	Aa	Bone		
4[c]	M	10 years	Rib	Aa	Bone		
5	F	13 years	Os ilium dextrum	Aa	Bone		
6[c]	F	14 years	Skull	Aa	Bone		
7	M	15 years	Skull	Aa	Bone		
8	M	Connatal	Skin	Ac	Skin (abdomen)	No treatment	No evidence of disease
9[c]	M	4 months	Skin	Ac	Skin (abdomen, groin)	A[d]	Relapse: skin Progression: skull (multifocal)
10	M	6 months	Skin	Ac	Skin (abdomen, face)	No treatment	Persistence of disease
11	M	3 years	Skull, ribs Humerus dexter Femur dexter et sinister	Ba	Bone	A	No evidence of disease
12	F	3 months	Lymph nodes Liver	Bb	Lymph node (cervical, inguinal)	B	No evidence of disease
13	M	4 months	Lumbal vertebrae Skin, gingiva Lung	Bb	Skin (abdomen)	B	No evidence of disease
14	F	6 months	Rib Skin, gingiva	Bb	Bone Skin (breast)	B,C	Delayed remission
15	F	10 months	Skull, humerus sin. Lymph node	Bb	Bone	B	No evidence of disease
16	M	6 years	Tumor of left sterno-clavicular joint (bone and soft tissue)	Bb	Tumor	B	No evidence of disease
17[c]	M	8 years	Two ribs, sternum Lymph nodes	Bb	Lymph node (cervical, Submandibular)	Other polychemotherapy	No evidence of disease
18[c]	M	15 years	Appendectomy scar	Bb	Scar	Other polychemotherapy	No evidence of disease
19	F	1 year	Os ilium (multifocal) Skin Hematopoietic system + dysfunction Liver + dysfunction Spleen	Bc	Skin (Abdomen, Face) Liver	C	No evidence of disease
20	M	1 year	Skin, gingiva Liver + dysfunction Lung + dysfunction	Bc	Skin	C	Persistence of disease
21	M	4 years	Skull (multifocal) Skin Hematopoietic system + dysfunction Retrobulbar tumors	Bc	Skin Retrobulbar tumor	C	Progression: lung, pancreas, liver + dysfunction

[a] See Table III.
[b] Biopsy specimens available for immunohistochemical analysis
[c] Patients that were not a priori included in the DAL-HX 83 study.
[d] A,B,C – different therapy branches, DAL-HX 83.

Table III.

Classification of HX (DAL-HX 83)	Therapeutic Management [35][a]
A. Localized disease	
a. Bone (one or two lesions in one bone)	Local management
b. Lymph node	
c. Skin	
B. Disseminated disease	
a. Multifocal bone lesions	Polychemotherapy, branch A
Two lesions in one bone	
Several bones involved	
b. Soft tissue (except Ab,Ac)	Polychemotherapy, branch B
Bone and soft tissue	
c. Disease with organ dysfunction	Polychemotherapy, branch C

[a] Six weeks of induction therapy (prednisone, vinblastine, etoposide — VP16) followed by 46 weeks maintenance therapy (6-mercaptopurine) interrupted by reinduction pulses (A = prednisone, vinblastine; B = prednisone, vinblastine, VP16; C = prednisone, vinblastine, MHD-methotrexate).

two groups (A = localized disease, B = disseminated disease), within the DAL-HX 83 staging protocol, on the basis of which the respective therapeutic regimen [35] was initiated (Tables II and III). Course of the disease, therapeutic efficiency, and other factors were monitored according the the DAL-HX 83 surveillance protocol. From 8 patients (6 males, 2 females; ages at diagnosis ranged from 3 months to 15 years) not included in the DAL-HX 83 study, sufficient clinical, laboratory, and histopathological data allowed them to be staged retrospectively according to the DAL-HX 83 protocol.

Biopsies Fresh biopsy specimens from different organs (Table II) taken for diagnostic light and electron microscopic examination were divided into several portions to allow immunohistologic visualization of the immunophenotype of the proliferating cell.

Monoclonal Antibodies The monoclonal antibodes used are listed in Table IV.

Tissue Processing For light and electron microscopy, tissue samples were processed according to standard methods. For immunohistologic studies, biopsy specimens were kept in RPMI 1640 up to 4 h and, subsequently, were snap-frozen in liquid nitrogen with quenching in 2-methylbutane and stored at −70°C until use. Four-micrometer aerial cryostat sections were mounted on glass slides, air-dried, acetone-fixed for 10 min at room temperature (RT), and stored at −20°C until use.

Immunoperoxidase Studies on Tissue Sections These studies were performed as previously described [28]. Briefly, air-dried cryostat sections were incubated for 45 min with 100 μl of the appropriately diluted monoclonal antibody. The antibody chain was prolonged by three consecutive 30-min incubation steps at RT using horseradish peroxidase (HRPO) rabbit anti-mouse Ig serum, HRPO swine anti-rabbit Ig serum, and, finally, HRPO rabbit anti-swine Ig serum [all from Dakopatts, Copenhagen, Denmark; working dilution 1:20 in 1:2 Tris–NaCl (pH 7.4):heat-inactivated human AB serum], with several Tris–NaCl (pH 7.4) washes after each incubation step. Sections were then subjected to the diaminobenzidine (DAB) reaction (6 mg of DAB in 10 ml Tris–HCl buffer, pH 7.6) for 10 min at RT and again washed in Tris–HCl (pH 7.6). After nuclear counterstaining with hematoxylin, sections were

Table IV. First-Step Monoclonal Antibodies Employed

Designation	Label	Working Dilution[a]	Ig subclass	Cellular Reactivity/ Antigenic Determinants	Reference and/or source
Anti-HLA-A,B	0	1:300	IgG1	All cells expressing class I MHC determinants	Hybritech
Anti-HLA-DR	0	1:300	IgG2a	All cells expressing HLA-DR	Becton/Dickinson
Anti-Leu-10	0	1:100	IgG1	All cells expressing HLA-DQ	Becton/Dickinson
VIC-Y1	0	1:100	IgG1	Human Ia cytoplasmic determinant located on multiple forms of invariant chain (γ, γ_2, γ_3)	[37], Inst. Immunology, Univ. Vienna, Austria
T29/33	0	1:100	IgG2a	Leukocyte common antigen (CD45)[b]	Hybritech
Anti-Leu-5b	0	1:100	IgG2a	T cells (CD2)[b]	Becton/Dickinson
Anti-Leu-1	0	1:100	IgG2a	T cells (CD5)	Becton/Dickinson
Anti-Leu-4	0	1:100	IgG1	T cells (CD3)	Becton/Dickinson
	PE	1:20			
VIT4	0	1:100	IgG2a	T cells Monocytes/macrophages LCs and related cells (CD4)	[38], Inst. Immunology, Univ. Vienna, Austria
VIT8	0	1:100	IgG1	T cells (CD8)	[38], Inst. Immunology, Univ. Vienna
OKT6	0	1:100	IgG1	Thymocytes	Ortho
	FITC	1:20		LCs and related cells (CD1)	
VIB-C5	0	1:100	IgM	B cells, mature granulocytes (CD24)	[39], Inst. Immunology, Univ. Vienna, Austria
Dako-C3bR	0	1:100	IgG	C3b receptor (CR1)	Dakopatts
VIM12	0	1:100	IgG1	C3bi receptor (CR3, CD11)	[40], Inst. Immunology, Univ. Vienna, Austria
Anti-Leu-M1	0	1:100	IgM	Myeloid cells (CD15) Some monocytes Sternberg–Paltauf–Reed cells Activated T cells	Becton/Dickinson
Anti-Leu-M3	0	1:100	IgG2b	Monocytes/macrophages (CDw14)	Becton/Dickinson
Ki-M1	0	1:4000	IgG1	Monocytes/macrophages IDCs	[31], Dept. Pathology, Univ. Kiel, West Germany
Ki-M4	0	1:2000	IgG3	Dendritic reticulum cells	[41], Dept. Pathology, Univ. Kiel, West Germany
Ki-M6	0	1:1000	IgG1	Monocytes/macrophages	[42], Dept. Pathology, Univ. Kiel, West Germany
Ki-M8	0	1:1000	IgG1	Monocytes/macrophages	Unpublished, Dept. Pathology, Univ. Kiel, West Germany
S-100	0	1:50	IgG1	S100 protein	Dakopatts
OKT9	0	1:200	IgG1	Transferrin receptor	Ortho
Anti-Tac	0	1:200	IgG2a	Interleukin-2 receptor (CD25)	[43], T. A. Waldmann, Bethesda, Maryland

[a] All monoclonal antibodies were diluted in PBS/3% bovine serum albumin.
[b] IUIS–WHO nomenclature for clusters of differentiation (CD) [44].

mounted in Kaiser's glycerol gelatin. Inhibition of endogenous peroxidase activity was performed according to Köller et al. [45].

Double-Labeling Immunofluorescence Studies on Tissue Sections Antibody binding was visualized using a two-step immunofluorescence protocol followed by a direct immunofluorescence staining procedure. Air-dried cryostat sections were incubated for 45 min with 100 μl of the appropriately diluted monoclonal antibody. They were washed three times in phosphate-buffered saline (PBS), overlayered (30 min, RT) with a 1:20 dilution of FITC-$F(ab')_2$ goat anti-mouse Ig antibody (Tago, Inc., Burlingame, California) or Texas Red AP rat anti-mouse IgG, F'2 (Jackson Immunoresearch Laboratories, Inc., Avondale, PA), and subjected to further PBS washes. After a subsequent 30-min (RT) exposure to PBS/5% normal mouse serum, the incubation chain was prolonged (45 min, RT) using the appropriately diluted FITC- or phycoerythrin (PE)-labeled second monoclonal antibody. After final PBS washes, sections were mounted with PBS/glycerin and examined under a Leitz-Ortholux II fluorescence microscope with an appropriate FITC and Texas Red/PE filter setting.

Controls Cryostat sections of human tonsils served as positive controls, allowing definition of the optimal working dilutions of the respective antibodies. Negative controls consisted of (1) omission of either the first or one of the subsequent antibody reagents; (2) substitution of primary antibody with normal mouse serum; and (3) substitution of primary antibody with an irrelevant monoclonal antibody of the same isotype.

RESULTS

Clinical Data Disease classification according to the DAL-HX 83 protocol [35], course of the disease, and therapeutic management are summarized in Table II.

Histopathology and Electron Microscopy Each biopsy specimen subjected to immunohistologic analysis displayed the histopathologic and ultrastructural characteristics of HX: predominantly large mononuclear cells with folded, partly grooved nuclei, abundant eosinophilic cytoplasm, and a varying number of Birbeck granules were accompanied by an admixed inflammatory infiltrate, composed mainly of eosinophils, small lymphocytes, and plasma cells.

Immunohistology Irrespective of the clinical presentation or the type of involved organ examined, virtually all large cells constituting HX lesions uniformly exhibited those antigenic determinants abundantly expressed by the entire group of LCs and related cells: cells were positive, with monoclonal antibodies defining class I and II alloantigens, T200 antigens, CD1 antigen, and S-100 protein. In general, the intensity of the reactivity toward a given antibody was similar in each tissue specimen despite a moderate variability between the individual cells in a given cryosection. Strongest staining was obtained by anti-HLA-A,B, anti-HLA-DR, anti-Leu-10, and OKT6 (Fig 1) followed by VIC-Y1 and anti-S-100. HX cells were uniformly unreactive with monoclonal antibodies to the interleukin-2 receptor, to the sheep red blood cell receptor (CD2), to pan-T antigens such as CD3 and CD5, to the T suppressor/cytotoxic cell-associated antigen CD8, and to B-cell antigens. All HX cells differed from "resting" LCs in that they displayed strong anti-CD4 reactivity (Fig 2) and uniformly expressed transferrin receptors. (Fig 3) [46].

In contrast to this rather homogenous immunohistochemical reactivity pattern of HX cells toward the monoclonal antibodies described so far, immunophenotypic differences between the prolifer-

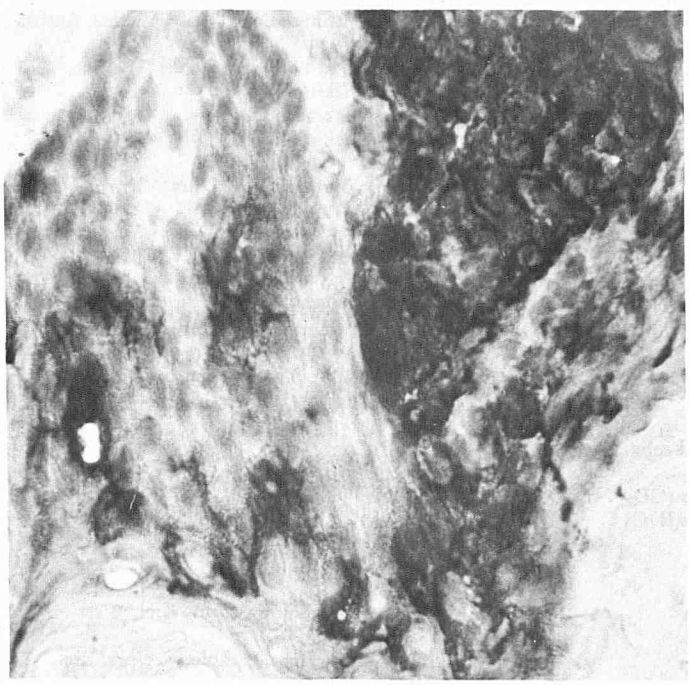

Figure 1. Photomicrograph of a cryostat section from a HX skin lesion stained by immunoperoxidase technique: anti-CD1 reactivity of numerous histiocytic cells infiltrating the papillary dermis and overlying epidermis (HX cells) as well as $CD1^+$ dendritic cells (LC) within the perilesional epidermis. ×250.

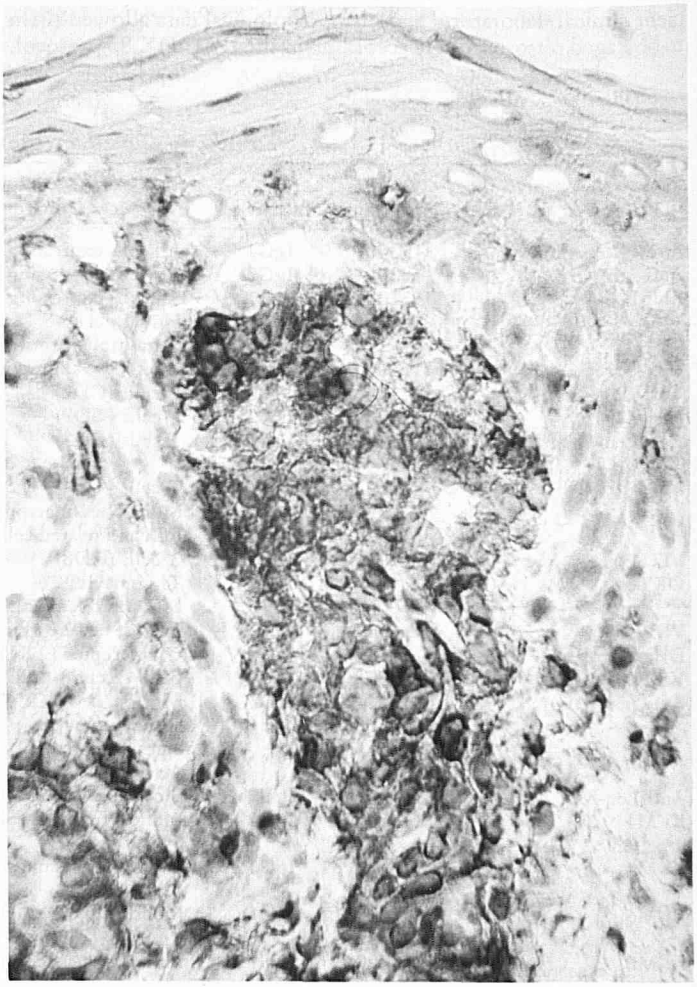

Figure 2. Photomicrograph of a cryostat section from an HX skin lesion stained by immunoperoxidase technique: anti-CD4 reactivity of numerous histiocytic cells infiltrating the papillary dermis (HX cells). Weak anti-CD4 reactivity within the epidermis can be attributed to either HX cells, LCs, or infiltrating T cells. ×250.

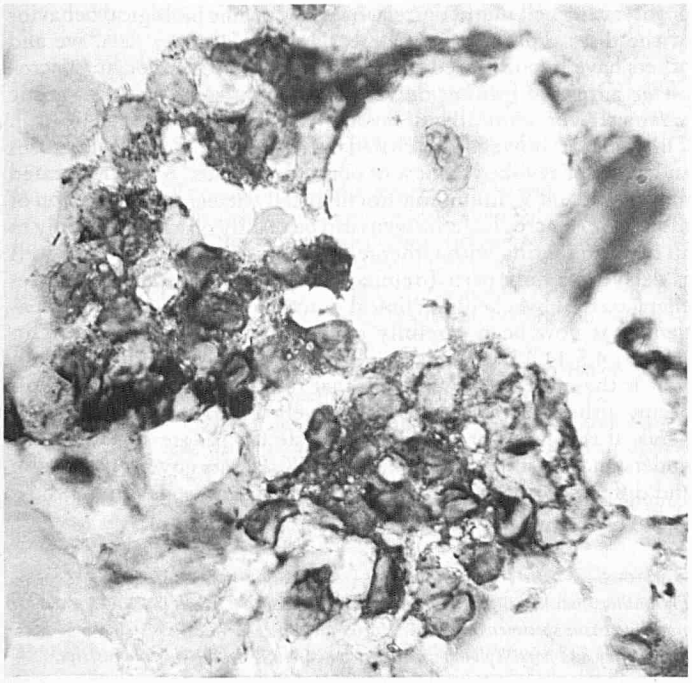

Figure 3. Photomicrograph of a cryostat section from an HX bony lesion stained by immunoperoxidase technique: transferrin-receptor expression by virtually all large cytoplasm-rich cells. ×250.

ating cells from different HX patients were observed when cryostat sections were exposed to antibodies to the C3b receptor (CR1; Dako-C3bR), to the C3bi receptor (CR3; VIM12), to IDC (Ki-M1), and to certain monocyte/macrophage antigens (CDw14, Ki-M6) (Table V). Immunofluorescence double labeling revealed moderate to strong CR1 and CR3 expression as well as weak to moderate staining with the antimacrophage antibodies anti-Leu-M3 and Ki-M6 of virtually all $CD1^+$, $CD3^-$ HX cells in five subjects (Nos. 4,5,8–10) suffering from either solitary bone or skin disease, in four subjects (Nos. 11 and 13–15), presenting with disseminated HX without organ dysfunction, and in three children (Nos. 19–21) suffering from disseminated disease with organ dysfunction. Staining intensity varied considerably betwen different patients but was uniform in a given subject (Table V). In subjects 8,9,11,20, and 21 comprising the entire clinical spectrum of HX, virtually all $CD1^+$ $CD3^-$HX cells displayed, in addition, strong and prominent immunolabeling with Ki-M1, a monoclonal antibody-recognizing IDC (Fig 4); exposure to anti-Leu-M1, Ki-M4, and Ki-M8 consistently yielded negative results.

DISCUSSION

In this study we have demonstrated immunophenotypic heterogeneity of HX cells in 21 children suffering from different clinical manifestations of HX. It appears that HX cells regularly express class I and II alloantigens, T200, CD1, and CD4 antigens, as well as S-100 protein. The concomitant presence of immunocytochemically detectable amounts of additional surface antigens (e.g., CR1 and CR3 receptors, myeloid differentiation antigens CDw14 and Ki-M6) was encountered on virtually all HX cells in 12 of 21 HX cases, 5 of which were further immunoreactive for Ki-M1. Thus, our results appear to divide the 21 HX cases into three distinct groups based on their immunochemically traceable expression of complement receptors as well as the CDw14, Ki-M6, and Ki-M1 antigens. Despite this immunophenotypic variability of HX cells, their immunological marker repertoire described is highly compatible with the phenotypic scope of LCs and related cells, thus supporting the contention that HX indeed represents a proliferative disorder of this particular cell system.

Comparison of antigen expression profiles of HX cells with a

Table V. Reactivity of HX Cells With Anti-Monocyte/Macrophage Antibodies

Subject	Classification DAL-HX83	Biopsy	CR1 (Dako-C3bR)	CR3 (VIM12)	Anti-Leu-M3	Anti-Leu-M1	Ki-M1	Ki-M4	Ki-M6	Ki-M8
1	Aa	Bone	0[a]	0	0	0	0	0	0	0
2	Aa	Bone	0	0	0	0	0	0	0	0
3	Aa	Bone	0	0	0	0	0	0	0	0
4	Aa	Bone	++	++	+	0	0	0	+	0
5	Aa	Bone	++	++	+	0	0	0	+	0
6	Aa	Bone	0	0	0	0	0	0	0	0
7	Aa	Bone	0	0	0	0	0	0	0	0
8	Ac	Skin	+++	+++	+	0	+++	0	++	0
9	Ac	Skin[b]	+++	+++	+	0	+++	0	++	0
			+++	+++	+	0	+++	0	++	0
10	Ac	Skin[b]	++	++	+	0	0	0	+	0
			++	++	+	0	0	0	+	0
11	Ba	Bone	++	+++	++	0	+++	0	++	0
12	Bb	*Lymph node*[b]	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
13	Bb	Skin	++	++	+	0	0	0	++	0
14	Bb	Bone	++	++	+	0	0	0	+	0
		Skin	++	++	+	0	0	0	+	0
15	Bb	Bone	++	++	+	0	0	0	+	0
16	Bb	Tumor	0	0	0	0	0	0	0	0
17	Bb	Lymph node[a]	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
18	Bb	Scar	0	0	0	0	0	0	0	0
19	Bc	Skin[b]	+++	+++	++	0	0	0	++	0
			+++	+++	++	0	0	0	++	0
		Liver	+++	+++	++	0	0	0	++	0
20	Bc	Skin	+++	+++	++	0	+++	0	++	0
21	Bc	Skin	+++	+++	+	0	+++	0	+	0
		Tumor	+++	+++	+	0	+++	0	+	0

[a] Reactivity: 0, none; +, weak; ++, moderate; +++, strong.
[b] Tissue specimens from two different biopsy sites were evaluated.

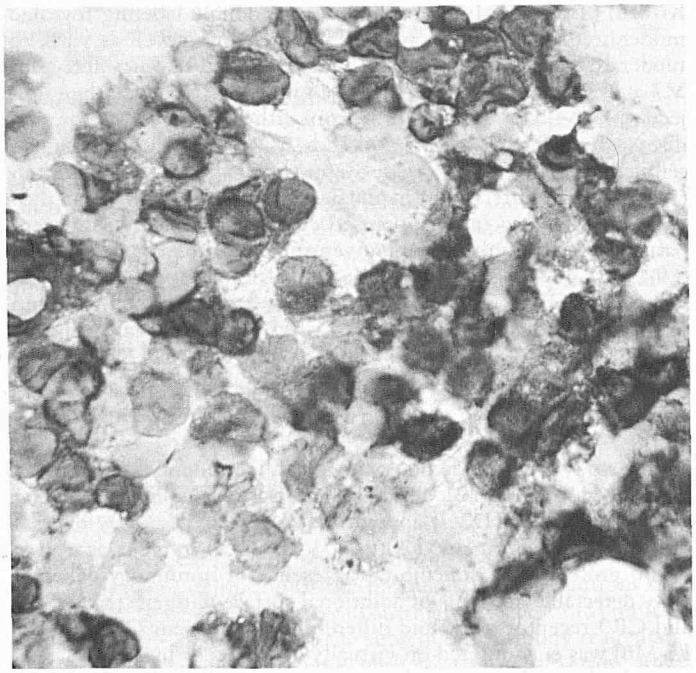

Figure 4. Photomicrograph of a cryostat section fron an HX bony lesion stained by immunoperoxidase technique: Ki-M1 reactivity of virtually all large cytoplasm-rich cells. ×250.

given phenotype known to be expressed by LCs and related cells suggests that Ki-M1-reactive HX cells correspond to IDCs [31], while strongly $CD4^+$ HX cells that lack immunocytochemically detectable amounts of complement receptors and monocyte/macrophage antigens share their phenotype with epidermal LCs under certain disease conditions [28]. An immunophenotype similar to that of $CR1^+$, $CR3^+$, $CDw14^+$, and $Ki\text{-}M6^+$ HX cells has recently been described for a subset of mononuclear cells in peripheral blood of extensively burned individuals and in human cord blood [15,47]. These circulating cells are thought to represent LC precursors traveling from their bone marrow origin to their epidermal residence [15,47]. Immunolabeling with anti-complement receptor and anti-monocyte/macrophage reagents has been further described for cultured human LCs [34] (our observation); these cells, however, differ from HX cells by their lack of CD4 (our observation) and acquisition of interleukin-2 receptor expression [33].

Among the many possible factors regulating gene expression in a given cell, it has been postulated that it is the specific epidermal microenvironment that provides factors responsible for the specific antigen expression pattern of epidermal LCs [32]. If a similar phenomenon should be operative for HX cells, that is, that the organ-specific milieu determines the immunophenotype of the proliferating cell, one would have to expect that HX cells displaying the phenotype of IDCs exclusively populate T-cell-dependent areas of peripheral lymphoid organs, while HX cells constituting skin lesions should display the antigenic repertoire of either resting or activated epidermal LCs. Our results, however (i.e., IDC-specific Ki-M1 expression by HX cells in HX skin disease; lack of monocyte/macrophage antigenic determinants on HX cells proliferating within lymph nodes; uniform HX cell phenotypes in various organs in one given patient), demonstrate that the tissue-specific microenvironment alone does not suffice to regulate antigen expression characteristics of HX cells. Further studies will have to elucidate whether cell transformation through viral elements or oncogenes contributes to the phenotypic variability of HX cells. In this context we should mention that although we carefully screened for the presence of viral particles, we could not detect ultrastructural signs of viral infection of HX cells.

It is tempting to speculate that a certain immunophenotype of the proliferating cell might correlate with a specific biological behavior of the disease process. On the basis of preliminary data, we and others have hypothesized that the abundance of monocyte/macrophage antigenic features on HX cells might serve as a prognostic parameter for clinically unfavorable disease outcomes [48–50]. This concept, however, was based on a very limited number of cases and has to be revoked in view of our recent results. As demonstrated inTables II and V, immunohistochemically detectable expression of monocyte/macrophage antigens can be readily observed not only in all cases presenting with either relapse or disease progression as well as delayed or only partial remission, but also in a number of cases characterized by a benign clinical outcome. Remission in these patients has now been carefully controlled longer than 2 years in subjects 4,5,11,13,15, and 19 and longer than 12 months in patient 8. It is therefore rather unlikely that relapse of disease would still occur, although we cannot definitively exclude this possibility. Thus, at this point, it seems that despite the progress made in our understanding of the histogenesis of HX, factors governing growth and differentiation patterns of the proliferating cells remain to be elucidated.

The authors thank Dr. J. Hutterer and Dr. A. Zoubek for their patient care and for providing tissue specimens, Mrs. A. Harrer for expert technical assistance, and Mrs. Hebe Jeffrey and Mrs. Sylvia Ehrenhofer for carefully typing this manuscript.

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